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Scanning electron microscopy of the leaf epicuticular waxes of the genus *Gethyllis* L. (Amaryllidaceae) and prospects for a further subdivision

C Weiglin

Technische Universität Berlin, Herbarium BTU, Sekr. FR 1-1, Franklinstrasse 28-29, D-10587 Berlin, Germany
e-mail: CWeiglin@csi.com

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The leaf epicuticular wax ultrastructure of 32 species of the genus *Gethyllis* are for the first time investigated and discussed. Non-entire platelets were observed in 12 species, entire platelets with transitions to granules in seven species, membranous platelets in nine species and smooth layers in eight species. Only *Gethyllis transkarooica* is distinguished by its transversely ridged rodlets. The occurrence of transversely ridged rodlets is conspicuous and is interpreted as being convergent. In three species wax dimorphism was discovered, six species show a somewhat rosette-like orientation of non-entire or entire platelets and in four species a tendency to parallel orientation of non-entire or entire platelets was evident. It seems that *Gethyllis*, from its wax morphology, is highly diverse and deserves further subdivision.

Introduction

The outer epidermal cell walls of nearly all land plants are covered by a cuticle consisting mainly of cutin, an insoluble lipid polyester of substituted aliphatic acids and long chain primary and secondary alcohols (Holloway 1982). The cuticles of the majority of seed plants are covered with epicuticular wax secretions in the form of wax layers and crusts or epicuticular crystalloids or both (surveys in Martin and Juniper 1970, Barthlott and Wollenweber 1981, Cutler *et al.* 1982, Jeffree 1986, Barthlott *et al.* 1998).

Since the first SEM-studies were published (Amelunxen *et al.* 1967), it became evident that epicuticular wax crystalloid types are a relevant systematic and taxonomic character (Barthlott and Wollenweber 1981, Jeffree 1986, Frölich and Barthlott 1988, Barthlott 1990, 1994, Barthlott and Theisen 1998, Barthlott *et al.* 1998). It has been shown that the respective type of epicuticular waxes (and other cuticular sculpturing) is genetically determined (Cutler and Brandham 1977, Barthlott and Wollenweber 1981, Cutler 1982) and is often specific for a taxon at different levels: a genus (i.e. *Eucalyptus* L. Héritier: Hallam and Chambers 1970, *Nelumbo* Adans.: Barthlott *et al.* 1996), a family (i.e. Poaceae: Baum *et al.* 1980, Tulloch 1981, Fabaceae: Ditsch *et al.* 1995) or higher taxonomic groups (Frölich and Barthlott 1988, Hennig *et al.* 1994, Ditsch and Barthlott 1997).

Some environmental influences and infraspecific variations in the structure and development of epicuticular waxes (Juniper 1960, Leigh and Matthews 1963, Hall *et al.* 1965, Hallam 1970, Rentschler 1974, Baker 1974, 1982) and convergent shapes of waxes based on different chemical composition, however, can sometimes occur mainly in some single species among larger taxa, cultivated plants, varieties and mutants (Juniper 1960, Leigh and Matthews 1963, Hall *et al.* 1965, Hallam 1970, Rentschler 1974, Baker 1974, 1982, Reed and Tukey 1982) and must therefore be taken into account (Meusel *et al.* 1994). On the other hand, environmental influences and modifications of epicuticular wax ultrastructure were observed. Although these influences could alter the degree of the wax deposits (from either very dense to less dense or totally absent) or its chemical composition (Juniper 1960, Hall *et al.* 1965, Hallam 1970, Baker 1974, Rentschler 1974, Reed and Tukey 1982), only in rare cases is the crystalloid type itself changed (Baker 1982). Therefore, in most cases the ultrastructure of epicuticular wax crystalloids has been shown to be a significant systematic character in higher and lower level taxonomy (Jeffree 1986, Engel and Barthlott 1988, Fehrenbach and Barthlott 1988, Frölich and Barthlott 1988, Barthlott 1990, 1994, Hennig *et al.* 1994, Theisen and Barthlott 1994, Ditsch *et al.* 1995, Wilhelmi and Barthlott 1997, Barthlott and Theisen 1998, Barthlott *et al.* 1998).

Gethyllis L. possesses some unique features which are exceptional within the Amaryllidaceae. It is the only genus where 6, 12, 18 or more stamens (sometimes connate), a spiral arrangement of leaves and, together with *Apodolirion*, a subterranean ovary occur (Dahlgren, Clifford and Yeo 1985, Meerow 1995, Müller-Doblies and Müller-Doblies 1996, Meerow and Snijman 1998, Snijman 2000). From a systematic viewpoint *Gethyllis* is usually allied to the tribe *Haemantheae* which possessses many supposed primitive features (Björnstad and Friis 1972). Its exact position, however, is still under discussion. Dahlgren, Clifford *et al.* (1985)

considered *Gethyllis* (and *Apodolirion* Baker) as possibly being related to the basal tribe Haemantheae while Meerow (1995) assigned it to this tribe. Müller-Doblies and Müller-Doblies (1996) and Meerow and Snijman (1998) placed it in the tribe Gethyllideae. In an updated phylogenetic analysis of the Amaryllidaceae, however, the tribe Gethyllideae finds no molecular support (Meerow *et al.* 1999).

Since the first revision of the genus by Baker (1885) who recognised nine species, new species were added until 1933 and later by Müller-Doblies (1986) so that, today, it comprises about 32 species all located in the winter rainfall areas and also the Karoo of southern Africa (Burtt 1970, Snijman 2000). H Bolus, using a single character, was the first who attempted to subdivide the genus *Gethyllis* into the sections *Clinostylis* which included species with a long, exserted and declined style and *Orthostylis* which included species with a straight and (comparatively) short style (MacOwan and Bolus 1881). Baker (1885), however, made reference in his monograph to this character in his key, but failed to mention the section names. Nearly 100 years later, based on his preliminary investigations, Müller-Doblies (1982, 1986) suggested a further subdivision of *Gethyllis*, i.e. into two subgenera with ten series (Prof Dr Müller-Doblies, pers. comm.). For systematic and taxonomic purposes, the epicuticular waxes of 32 species of the genus *Gethyllis* L. were studied by high resolution SEM with a view to facilitating a better understanding of the genus.

Many proposals for the classification of the different wax crystalloid types have, over a period of time, been published (De Bary 1871, Amelunxen *et al.* 1967, Wilkinson 1979, Barthlott and Wollenweber 1981, Baker 1982, Jeffree 1986). The most recent typification by Barthlott *et al.* (1998) shall form the basis for this presentation.

Materials and Methods

Young leaves were harvested from the living collection BTU (Berlin-Dahlem) (Table 1) and pieces of about 3 square mm were immediately fixed for 4.5 hours with 2.5% glutaraldehyde. The leaves were fixed, infiltrated, and dehydrated using the glycerol infiltration method described by Ensikat and Barthlott (1993). After dehydration the samples were mounted on commercial SEM-stubs with double-adhesive tape and stored for two weeks or longer in an desiccator with silica gel. The specimens were coated with gold using a cooled sputter coater SCD 030 (Balzers Union, FL 9496 Balzers) in a 0,05mbar Argon atmosphere (10mA), with a thickness of 7nm, measured by a Cressington MTM oscillation-quartz-system (corresponding to about 1.5–2min sputtering time), observed with a Hitachi S-520 Scanning Electron Microscope with 20kV accelerating voltage (0,1nA) and photographed on Kodak plus-X 125 Professional film for black and white prints. The upper and lower leaf surfaces of all species were investigated. Illustrations in Figures 1–12 are drawn from the glycerol-infiltrated material and listed in the first column of Table 1 under 'species'. Specimens from other localities, either air-dried or fresh, were used only as controls. Herbarium voucher specimens are kept in herbarium BTU under their collection numbers.

Results

The main wax types found in the genus *Gethyllis* are described briefly in section A using the terminology of Barthlott et al. (1998) and are illustrated in Figures 1–12. In section B the species are listed in alphabetical order and, whenever a characteristic pattern or assembly of their wax type is present, it is described in greater detail. In Table 2 the investigated species are summarised according to their respective wax types. Unless otherwise stated, no differences between upper and lower leaf surfaces were found and the wax crystalloids are non-oriented at the cell surface. For description purposes the density of the wax layer was defined as 'loose' (approximately 30–50% of the surface visible), 'dense' (10–30% of the surface visible) and 'very dense' (surface not visible). If two wax types were observed on one cell surface the term wax dimorphism is used. In this paper the terms 'platelets' (instead of scales) and 'crystalloids' (instead of crystals / crystallites) are preferred (cf. discussion in Barthlott *et al.* 1998). A few unpublished names of species/subspecies were used in the text. To highlight this, these names were marked with parentheses followed by their collection numbers.

A. Wax crystalloid types in Gethyllis

Smooth layers (Figure 1): According to Barthlott *et al.* (1998), continous wax coverings are normally less than 1µm thick and lack any visible surface sculpturing. Species without wax crystalloids are described by this term in this paper. Because no fissures or cracks were observed in any of the species investigated, however, this term is purely speculative. But it can be assumed that the thin wax coverings, hardly detectable in SEM, represent the obligate outermost border of the cuticle of presumably all land plants (cf. Hallam 1982, Jeffree 1986, Barthlott *et al.* 1998).

Granules (Figure 5): Irregular, mostly isodiametric, often rounded crystalloids, ranging from very small to moderately large. In this paper this type is only observed as a transitional form to entire platelets (see below).

Non-entire (= irregular) platelets (Figures 2 and 9): Flat crystalloids, more or less irregular in shape and with irregular margins, usually 1–10µm high, protruding ± perpendicularly from the surface.

Entire platelets (Figures 7 and 8): Like the former type but with entire margins and regular shape, usually 1–3µm high and often semicircular.

Membranous platelets (Figures 3, 4 and 12): Flat, membranous crystalloids, usually interconnected, often forming a meshwork of threadlike extensions, protruding from the surface at various angles from flat to upright, margins normally very irregular.

Transversely ridged rodlets (Figure 6): Wax rodlets of variable length with ridges perpendicular to their axes, oriented ± perpendicular to the surface. The crystalloids have irregular cross sections and are sometimes hollow.

Table 1: *Gethyllis* species together with cultivation and collection number, collector name, grid number and locality. In most cases 3–4 origins were investigated. Herbarium voucher specimens are kept in herbarium BTU under collection number. Unpublished names of species or subspecies are marked with parentheses

Species	Cultivation number	Collector and Collection number	Grid number	Locality
afra	6620.1,1	U.&D.M-D. 86033	CAPE.- 3319CB (Worcester)	Worcester: West next to Polhillia waltersii Nature reserve
	2283.0/1	U.&D.M-D. 78106	CAPE.-3420BA (Bredasdorp)	Buffeljagsriv. coloured school. School ground
	1949.0/1	U.&D.M-D. 78028	CAPE.-3219CC (Wuppertal)	Theerivier 25km S of Citrusdal: Gravel bank of the river
	2291.0/1	U.&D.M-D. 78111	CAPE.-3320CC (Montagu)	Drew station. Railway terrain, degraded by rubbish dump
afra (ssp. *philosophica*)	3372.0/1	U.&D.M-D. 79236	CAPE.-3118DB (Vanrhynsdorp)	Zandkraal: Gifberg road, 2.7km S of turn off to Z
barkerae	3443.0/-	U.&D.M-D. 03443 (PLP.1025)	CAPE.-3119CD (Calvinia):	Botterkloof: Top of pass, E of road towards escarpment
	1694	U.&D.M-D. 77067	CAPE.-3118DC (Vanrhynsdorp)	Trawal: 17km S of Klawer, on road N7. SE-facing slope Fonteintjies Kloof
	2097.0/2	U.&D.M-D. 78063	CAPE.-3118DC (Vanrhynsdorp)	Heerenlogement :TMS rock pockets
	2098.0/1	U.&D.M-D. 78063	see above	see above
britteniana	2100.0/4	U.&D.M-D. 78063	see above	see above
	1693/11,12-	U.&D.M-D. 77066	CAPE.-3118DC (Vanrhynsdorp)	Voorspoed: 2km S of Klawer, W of N7
	2121.0/3	U.&D.M-D. 78064	CAPE.-3118DC (Vanrhynsdorp)	Heerenlogement: About 6km on the road to Vredendal
	3308.0/2	U.&D.M-D. 79227	CAPE.-3017DB (Hondeklipbaai)	Waterklip: W-slope, 200m on Sabies Farm, SW of Garies
	3323.0/r	U.&D.M-D. 79229	CAPE.-3018CD (Kamiesberg)	NE of road N7: 1.8km on Bitterfontein /Pofadder road
(*calcicola*)	6020/2	U.&D.M-D. 84028	CAPE.-3420BD (Bredasdorp)	Potberg Estates: 1.5km E of Witwater homestead
campanulata	6751	U.&D.M-D. 86046	CAPE.-3119AC (Calvinia)	Vanrhynspas: N of the pass
	1978.0/1	U.&D.M-D. 78034	CAPE.-3119AC (Calvinia)	Farm Grasberg: NW of Nieuwoudtville
	2184.0/1	U.&D.M-D. 78083	CAPE.-3119AC (Calvinia)	Glen Lyon Estates: SE of Nieuwoudtville.
cavidens	2137.2	U.&D.M-D. 78067	CAPE.-3118DA (Vanrhynsdorp)	Aties: 0.5km to Aties, E of the road to Klawer
ciliaris	2845.2,1-2,7	U.&D.M-D. 79130	CAPE.-3219CC (Wuppertal)	Theerivier: Farm 25km S of Citrusdal, right river bank of Olifantsriver
	4376	U.&D.M-D. 80117	CAPE.-3219AA (Wuppertal)	Elizabethfontein: 1.5km W from Clanwilliam/Calvinia road
grandiflora	3106.2,2	U.&D.M-D. 79185	CAPE.-2917DB (Springbok)	Nababiep: Klipdam Golf Course; 3.5km W of Okiep
gregoriana	2152.1,1	U.&D.M-D. 78072	CAPE.-3119AC (Calvinia)	Glen Lyon Estates: SE of Nieuwoudtville, loamy paddock W of Rocky Ridge
	6785	U.&D.M-D. 86051	CAPE.-3219AC (Wuppertal)	Algeria: Widdringtonia forest, slope opposite forest station
	1951.0/1	U.&D.M-D. 78028	CAPE.-3219CC (Wuppertal)	Theerivier: 25km S of Citrusdal
	2194.0/1	U.&D.M-D. 78084	CAPE.-3118DB (Vanrhynsdorp)	Zandkraal: 11km SSE of Vanrhynsdorp, at turn off to the farm
	4437.0/1	U.&D.M-D. 80127	CAPE.-3119CD (Calvinia)	Botterkloof: Top of pass
hallii	3350	U.&D.M-D. 79233	CAPE.-3118CB (Vanrhynsdorp)	Liebendal: SW of main entry
	3350.0/2	U.&D.M-D. 79233	see above	see above
heinzeana	8515/1,1-1,4	U.&D.M-D. 90119	CAPE.-3018CD (Kamiesberg)	NE of road N7: 1.8km on Bitterfontein /Pofadder road
lanuginosa	1676/8,3-	U.&D.M-D. 77063	CAPE.-3118DB (Vanrhynsdorp)	Zandkraal: 11km SSE of Vanrhynsdorp, at turn off to Zandkraal
	2124.0/1	U.&D.M-D. 78065	CAPE.-3118CB (Vanrhynsdorp)	Liebendal: ca. 10km NW of Vredendal
lata	1607.0/8	U.&D.M-D. 77052	CAPE.-3119AD (Calvinia)	Soetwater: 2km W of turn off to Clanwilliam
	4433.0/r	U.&D.M-D. 80126	CAPE.-3119CB (Calvinia)	Pylriet: 5km W of Botterkloof road
linearis	1670/3,1	U.&D.M-D. 77062	CAPE.-3118DB (Vanrhynsdorp)	Vanrhynsdorp: 6km on the road to Nieuwoudtville
	1609.0/5	U.&D.M-D. 77052	CAPE.-3119AD (Calvinia)	Soetwater: 2km W of turn off to Clanwilliam
	1641.0/3	U.&D.M-D. 77057	CAPE.-3119AC (Calvinia)	Drifontein road: 6.5km N of Nieuwoudtville/Calvinia road
	3862.0/2	U.&D.M-D. 80071	CAPE.-3119AD (Calvinia)	Hantamsrivier: between Calvinia and Loeriesfontein
longistyla	6117.2,1-2,2	U.&D.M-D. 84052	CAPE.-3222AD (Beaufort West)	Karoo: National Park, near hyena trap (N15C)

Table 1: continued

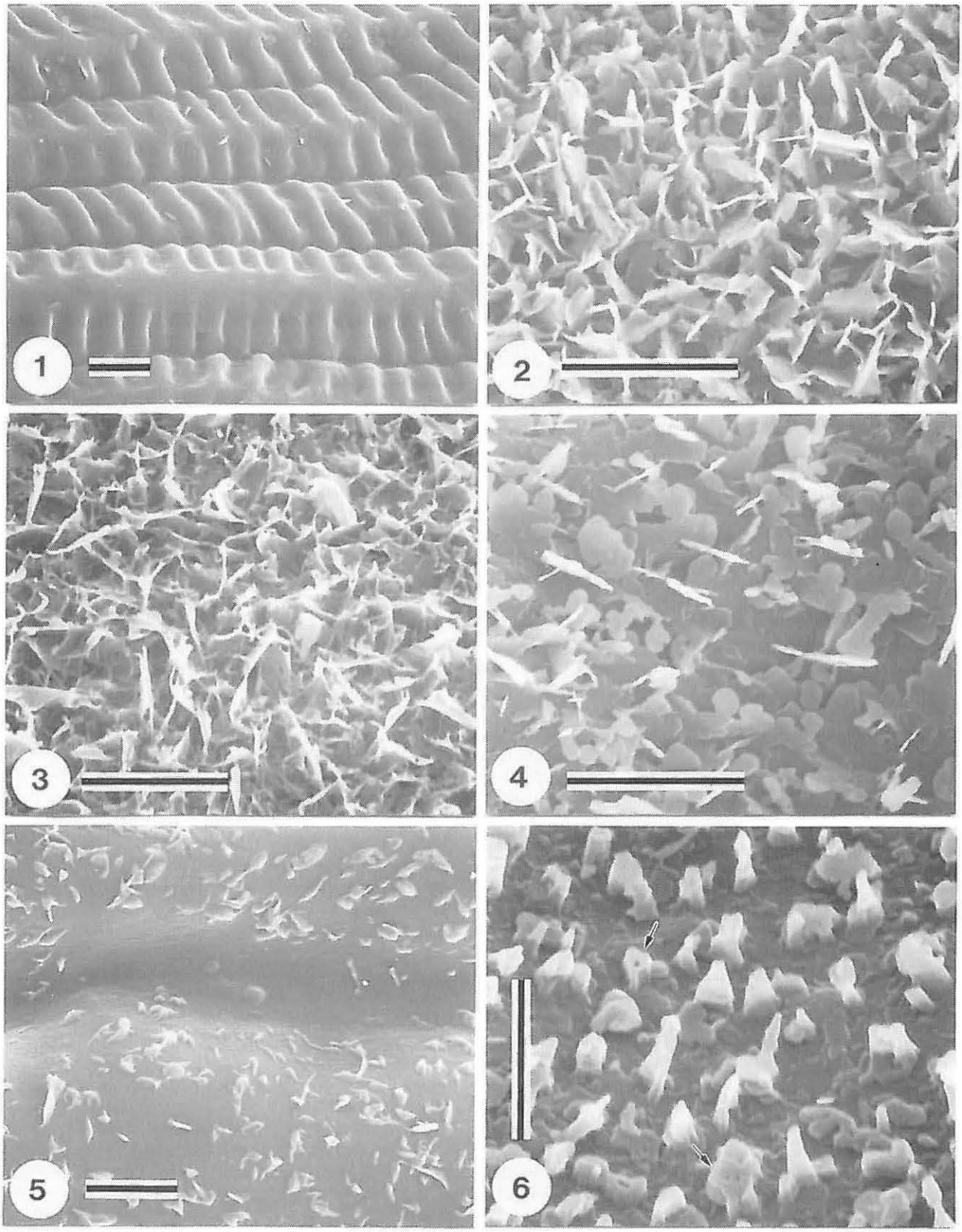
Species	Cultivation number	Collector and Collection number	Grid number	Locality
multifolia	3581	U.&D.M-D. 80025	CAPE.-3320BA (Montagu)	Matjesfontein: 1km on the way to Whitehill
	3581.0/3	U.&D.M-D. 80025	see above	see above
	1500.0/3	U.&D.M-D. 77035	CAPE.-3319BC (Worcester)	De Doorns: Airport, Hexrivervalley, near the hangar and the airstrip
	5321.0/1	U.&D.M-D. 82145	CAPE.-3320BC (Montagu)	Ezelsfontein: 12km E of Fisantekraal
namaquensis	7448	U.&D.M-D. 88144	NAMIBIA.-2716CB (Witputz)	Aurus Mts.: E of summit (Diamond area no. 1)
	2804/1,3	U.&D.M-D. 79110	CAPE.-2816BD (Oranjemund)	Helskloof: 1km S of top
oligophylla	5155/4	U.&D.M-D. 82118	CAPE.-3218BB (Clanwilliam)	Langkloof: 17km N of Clanwilliam at Mt. Synnott
	4350.0/r	U.&D.M-D. 80115	CAPE.-3218BB (Clanwilliam)	Kleinkliphuis: 3.5km W of top of Pakhuispas
oliverorum	6200	U.&D.M-D. Homes s.n	??	
	2119	U.&D.M-D. 78063	CAPE.-3118DC (Vanrhynsdorp)	Heerenlogement
	2142	U.&D.M-D. 780681	CAPE.-3118DC (Vanrhynsdorp)	Doringr.bridge: SE of Klawer
pectinata	8422	U.&D.M-D. EGHO s.n	??	
	2000	U.&D.M-D.78039	CAPE.-3119AD (Calvinia):	Klipkoppie: On the farm Soetwater
roggeveldensis	6676/3	U.&D.M-D. 86040	CAPE.-3220AD (Sutherland)	Voelfontein: E of road, 7km from t.o. to Calvinia
	1857.0/1	U.&D.M-D. Hanek 2355	CAPE.-3119DA (Calvinia)	Westelike voetheuvels van Rebunieberg
	3761	U.&D.M-D. 80055	CAPE.-3119BD (Calvinia)	Downes: E of Calvinia, SE corner of Hantamsberg
setosa	5350/3	U.&D.M-D. 82149	CAPE.-3320BB (Montagu)	Laingsburg: 13.5km NW of N1 on Sutherland road
	5350.0/1	U.&D.M-D. 82149	see above	see above
spiralis	5286.1,...	U.&D.M-D. 82139	CAPE.-3320AD (Montagu)	Witwaterspoort: 6km S of Konstabel
	5757.0/r	U.&D.M-D. Lavr. 20949	CAPE.-3321DA (Ladismith)	Rooibergpas: near Calitzdorp
transkarooica	6205/1,1-	U.&D.M-D. 84104	CAPE.-3319AB (Worcester)	Farm Slagboom: on Agter Witzenberg road, 1km E of farmhouse
uteana	5245.1,1-1,2	U.&D.M-D. 82136	CAPE.-3320BA (Montagu)	Matjesfontein: 1km on the road to Driekop from N2
	3550.0/1	U.&D.M-D. 80022	CAPE.-3320BA (Montagu)	Ghaapkop: (=Ngaapkop) near Matjesfontein
	3579.0/2	U.&D.M-D. 80025	CAPE.-3320BA (Montagu)	Matjesfontein: 1km on the way to Whitehill
uteana (subsp. *glauca*)	5278.5-6	U.&D.M-D. 82139	CAPE.-3320AD (Montagu)	Witwaterspoort:: 6m S of Konstabel
	5278.0/*	U.&D.M-D. 82139	see above	see above
undulata	2844.0/1	U.&D.M-D. 79129	CAPE.-3218BB (Clanwilliam)	Seekoerivier: 12km N of Clanwilliam on N7
verrucosa	1608	U.&D.M-D. 77052	CAPE.-3119AD (Calvinia)	Soetwater: 2km W of turn off to Clanwilliam
	1608.0/3	U.&D.M-D. 77052	see above	see above
	1643	U.&D.M-D. 77058	CAPE.-3119AC (Calvinia)	Nieuwoudtville: 11km on the road to Loeriesfontein
verticillata	6753.2	U.&D.M-D. 86046	CAPE.-3119AC (Calvinia)	Vanrhynspas: N of the pass.
	3017.0/-	U.&D.M-D. 79172	CAPE.- 2817CD (Vioolsdrif)	32.5km from Steinkopf/Port Nolloth on road to Eksteenfontein
	3094.0/1	U.&D.M-D. 79185	CAPE.-2917DB (Springbok)	Nababiep: Klipdam Golfe Course; 3.5km W of Okiep
villosa	1625/7,23-	U.&D.M-D. 77054	CAPE.-3119AC (Calvinia)	Glen Lyon Estates: SE of Nieuwoudtville
	3317.0/1	U.&D.M-D. 79229	CAPE.-3018CD (Kamiesberg)	NE of road N7, 1.8km on Bitterfontein/Pofadder road
	3738.0/2	U.&D.M-D. 80050	CAPE.-3219DD (Wuppertal)	Karoo: 166km S of Calvinia
	5489.0/1	U.&D.M-D. 82166	CAPE.-3119CA (Calvinia)	Lokenburg: koppie 1km from entry
(*vogeliana*)	7308.5 SA_7	U.&D.M-D. 88112	CAPE.-3017DB (Hondeklipbaai)	Garies: 4.5km NW along N7

B. Wax crystalloid types of the investigated species

The following results are summarised in Table 2. Unpublished names of species/subspecies were marked with parentheses followed by their collection numbers.

G. afra L.: Very dense layer of membranous platelets on epidermal and guard cells as for example in *G. linearis* L. Bolus, *G. multifolia* L. Bolus (Figure 3) and *G. uteana* D.Müll.-Doblies (subsp. *glauca,* U.&D.M-D 82139) (see below).

G. afra L. (subsp. *philosphica*, U.&D.M-D 79236): Very dense layer of non-entire platelets on epidermal and guard cells with a distinct tendency to rosette-like orientation, each consisting of 5–15 platelets, as in *G. lata* L. Bolus, *G.*



Figures 1–6: Wax types found in species of *Gethyllis*. Ad/ab = adaxial/abaxial leaf surface **1.** *Gethyllis britteniana* (ab); smooth layer. **2.** *Gethyllis pectinata* (ad); non-entire platelets with crenate margins. **3.** *Gethyllis multifolia* (ab); membranous platelets. **4.** *Gethyllis heinzeana* (ad); membranous platelets. **5.** *Gethyllis longistyla* (ab); entire platelets with transition to granules. **6.** *Gethyllis transkarooica* (ab). transversely ridged rodlets, sometimes hollow (arrow). Scale bars: 1 = 20µm, 2 = 5µm, 3 = 5µm, 4 = 5µm, 5 = 5µm, 6 = 2µm

roggeveldensis D.Müll.-Doblies, and (*G. vogeliana*, U.&D.M-D 88112) (see below). *G. verticillata* R.Br. ex Herb. (Figure 7), a species with entire platelets, also showing this rosette-like orientation.

G. barkerae D.Müll.-Doblies: Smooth layer as in *G. britteniana* Baker (Figure 1), (*G. calcicola*, U.&D.M-D 84028), *G. oligophylla* D.Müll.-Doblies, *G. spiralis* (Thunb.) Thunb. and *G. undulata* Herb. (see below).

G. britteniana Baker (Figure 1): Smooth layer as in *G. barkerae* D.Müll.-Doblies and other species mentioned (see above). In this species, however, with much less and very small entire platelets on lower leaf surface.

(*G. calcicola*, U.&D.M-D 84028): Smooth layer as in *G. barkerae* D.Müll.-Doblies, *G. britteniana* Baker (Figure 1) and other species mentioned (see above).

G. campanulata L. Bolus (Figure 9): Dense layer of non-entire platelets in two distinct sizes on epidermal and guard cells (wax dimorphism). A larger size-class, partly with conspiciously elongated platelets and, in between, smaller platelets with transitions to granules. Slight tendency to parallel orientation of the larger platelets.

G. cavidens D.Müll.-Doblies: Loose layer of small entire platelets on epidermal cells, sometimes with transitions to granules, with distinct rosette-like orientation; guard cells almost completely without crystalloids. Same rosette-like orientation *in G. afra L.* (subsp. *philosphica*, U.&D.M-D 79236) (see above), *G. lata* L. Bolus, *G. roggeveldensis* D.Müll.-Doblies, *G. verticillata* R. Br. ex Herb. (Figure 7) and (*G. vogeliana*, U.&D.M-D 88112) (see below) but in these species with non-entire platelets.

G. ciliaris (Thunb.) Thunb.: Upper leaf surface with dense layer of membranous platelets with lobed margins as in *G. hallii* D.Müll.-Doblies, *G. heinzeana* D.Müll.-Doblies (Figure 4), and *G.uteana* D.Müll.-Doblies (Figures 11 and 12) (see below); guard cells with somewhat fewer crystalloids as in *G. heinzeana* D.Müll.-Doblies. Lower leaf surface having dense layer of non-entire platelets with crenate margins and with transitions to granules as for example in *G. pectinata* D.Müll.-Doblies (Figure 2) (see below). Guard cells almost completely without crystalloids.

G. grandiflora L. Bolus: Upper leaf surface without wax crystalloids (smooth layer) as in *G. barkerae* D.Müll.-Doblies, *G. britteniana* Baker (Figure 1) and other species mentioned (see above). Lower leaf surface with loose layer of non-entire platelets on epidermal cells; guard cells with distinctly fewer crystalloids.

G. gregoriana D.Müll.-Doblies: Very dense layer of non-entire platelets with crenate margins on epidermal and guard cells as for example in *G. pectinata* D.Müll.-Doblies (Figure 2) (see below).

G. hallii D.Müll.-Doblies: Upper leaf surface with dense layer of membranous platelets with lobed margins as in *G. ciliaris* (Thunb.) Thunb. (see above), *G. heinzeana* D.Müll.-Doblies (Figure 4) and *G. uteana* D.Müll.-Doblies (Figures 11 and 12) (see below); guard cells almost completely without crystalloids. Lower leaf surface with loose layer of non-entire platelets on epidermal and guard cells.

G. heinzeana D.Müll.-Doblies (Figure 4): Dense layer of membranous platelets with conspicuous lobed margins on epidermal cells as in *G. ciliaris* (Thunb.) Thunb., *G. hallii* D.Müll.-Doblies (see above) and *G. uteana* D.Müll.-Doblies (Figures 11–12) (see below); guard cells with distinctly fewer crystalloids.

G. lanuginosa Marl.: Loose layer of very small entire platelets with transitions to granules on epidermal and guard cells as for example in *G. longistyla* (Figure 5) (see below).

G. lata L. Bolus: Very dense layer of non-entire platelets on epidermal and guard cells with a distinct tendency to rosette-like orientation, each consisting of 5–15 platelets, as in *G. afra L.* (subsp. *philosphica*, U.&D.M-D 79236) (see above), *G. roggeveldensis* D.Müll.-Doblies and (*G. vogeliana*, U.&D.M-D 88112) (see below). *G. verticillata* R.Br. ex Herb. (Figure 7), a species with entire platelets, also showing this rosette-like orientation.

G. linearis L. Bolus: Very dense layer of membranous platelets on epidermal and guard cells as for example in *G. afra* L. (see above), *G. multifolia* L. Bolus (Figure 3) and *G. uteana* D.Müll.-Doblies (subsp. *glauca*, U.&D.M-D 82139) (see below).

G. longistyla H. Bolus (Figure 5): Dense layer of small entire platelets with triangular shape and with transitions to granules on epidermal cells; guard cells almost completely without crystalloids.

G. multifolia L. Bolus (Figure 3): Very dense layer of membranous platelets on epidermal and guard cells as for example in *G. afra* L., *G. linearis* L. Bolus (see above) and *G. uteana* D.Müll.-Doblies (subsp. *glauca*, U.&D.M-D 82139) (see below).

G. namaquensis (Schonl.) Oberm.: Loose layer of small entire platelets on epidermal cells, sometimes with transitions to granules, with distinct tendency for orientation into groups of parallel rows as in *G. setosa* (Figure 8) (see below); guard cells almost completely without crystalloids.

G. oligophylla D.Müll.-Doblies: Smooth layer as in *G. barkerae* D.Müll.-Doblies, *G. britteniana* Baker (Figure 1) and other species mentioned (see above).

G. oliverorum D.Müll.-Doblies: Loose layer of non-entire platelets with crenate margins on epidermal cells as it is for the dense layer in *G. pectinata* D.Müll.-Doblies (Figure 2) (see below); on guard cells only a few wax crystalloids.

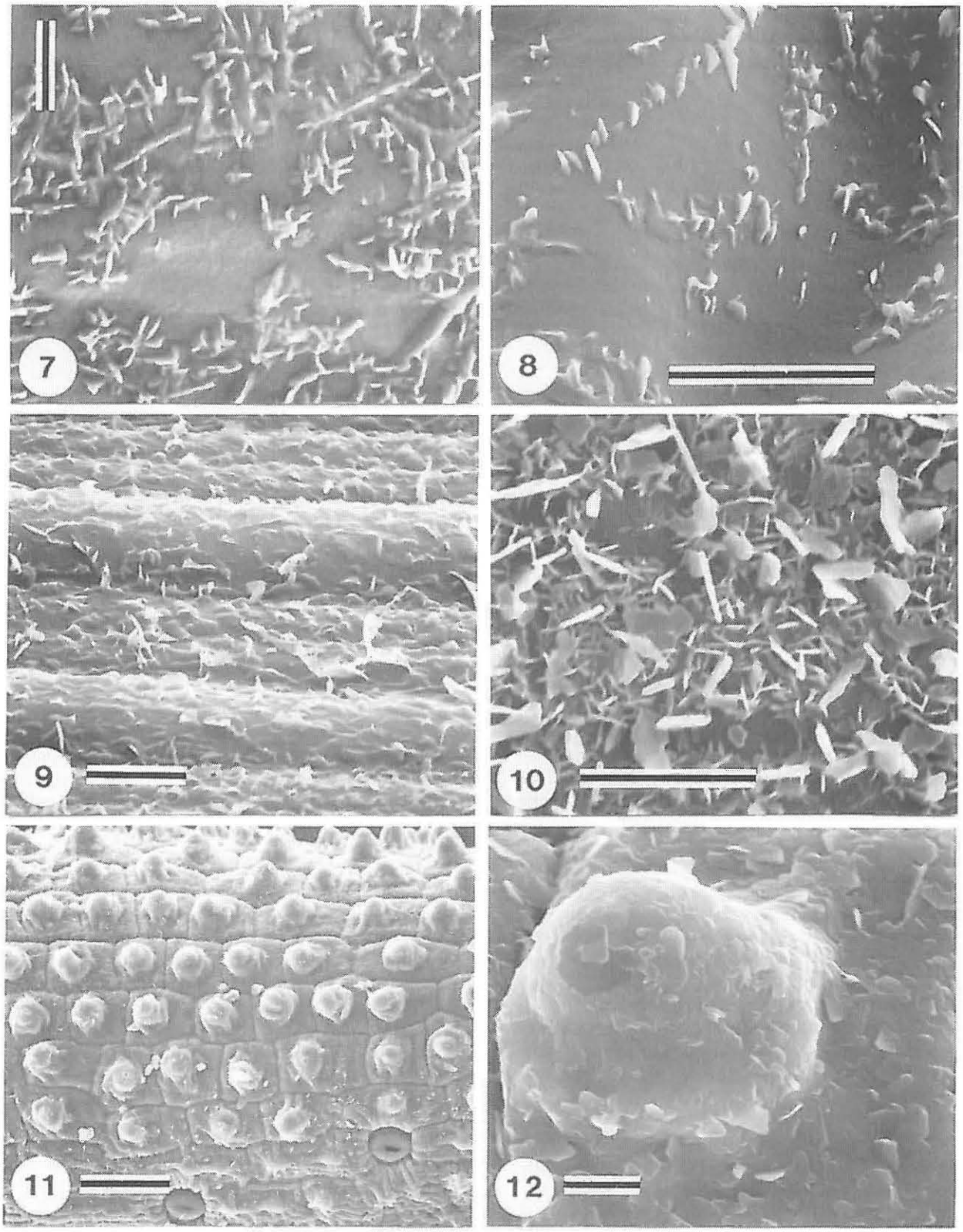
G. pectinata D.Müll.-Doblies (Figure 2): Dense layer of non-entire platelets with crenate margins on epidermal cells; guard cells with distinctly fewer crystalloids.

G. roggeveldensis D.Müll.-Doblies: Very dense layer of non-entire platelets on epidermal and guard cells with a distinct tendency to rosette-like orientation, each consisting of 5-15 platelets, as in *G. afra L.* (subsp. *philosphica*, U.&D.M-D 79236), *G. lata* L. Bolus (see above) and (*G. vogeliana*, U.&D.M-D 88112) (see below). *G. verticillata* R.Br. ex Herb. (Figure 7), a species with entire platelets, also showing this rosette-like orientation.

G. setosa Marl. (Figure 8): Loose layer of entire platelets on epidermal and guard cells. Platelets often appearing triangular in shape and groups of platelets oriented into parallel rows as in *G. namaquensis* (Schonl.) Oberm. (see above).

G. spiralis (Thunb.) Thunb.: Smooth layer as in *G. barkerae* D.Müll.-Doblies, *G. britteniana* Baker (Figure 1) and other species mentioned (see above).

G. *transkarooica* D.Müll.-Doblies: Dense layer of trans-



Figures 7–12: Wax dimorphism and orientation of crystalloids in species of *Gethyllis*. Ad/ab = adaxial/abaxial leaf surface. **7.** *Gethyllis verticillata* (ad); rosette-like orientation of small entire platelets. **8.** *Gethyllis setosa* (ad); entire platelets oriented into parallel groups or rows respectively. **9.** *Gethyllis campanulata* (ab); large and small non-entire platelets on one cell surface. **10.** *Gethyllis verrucosa* (ad); large plates and non-entire platelets on one cell surface. **11.** *Gethyllis uteana* (ad); peripheral leaf surface (left) with papilla-like epidermal cells and tabulated epidermal cells below (at the top). **12.** *Gethyllis uteana* (ad); papilla-like epidermal cell; membranous platelets with lobed margins. Scale bars: 7 = 5µm, 8 = 5µm, 9 = 5µm, 10 = 5µm, 11 = 50µm, 12 = 5µm

Table 2: Grouping of the species according to their respective wax types. If not otherwise indicated (ad/ab, see legend), no difference between upper and lower leaf surface was observed

Smooth layer	Membraneous platelets	Entire platelets	Non-entire platelets	Transversely ridged rodlets
G. oligophylla	G. ciliaris: ad [4) D]	G. lanuginosa [L]	G. gregoriana [VD]	G. transkarooica [L)]
G. spiralis	G. hallii: ad [5) D]	G. longistyla [5) D]	G. oliverorum [4) L]	
G. barkerae	G. heinzea [4) D]	**G. villosa** [1) L]	G. pectinata [4) D]	
G. uteana ⟷	G. uteana [D]	**G. namaquensis** [2) 5) L]	**G. campanulata** [1) D] ⟷	
(G. calcicola)	G. uteana (ssp. glauca) [VD]	**G. setosa** [2) L]	*G. roggeveldensis* [3) VD]	
G. britteniana	G. verrucosa [D] ⟷	G. verrucosa [D]	*G. afra* (ssp. *philosophica*) [3) VD]	
G. grandiflora: ad	G. linearis [VD]	*G. cavidens* [3) 5) L]	*G. lata* [3) VD]	
G. undulata	G. afra [VD]	*G. verticillata* [3) L]	(*G. vogeliana*) [3) D]	
	G. multifolia [4) D]		G. hallii: ab [D]	
			G. grandiflora: ab [4) L]	
			G. ciliaris: ab [D]	

Legend:

ad/ab = upper/lower leaf surface
1) = slight tendency to parallel orientation of crystalloids (**bold**)
2) = ± distinct parallel orientation of crystalloids (**bold**)
3) = rosette-like orientation of crystalloids (*italics*)
4) = guard cells with fewer crystalloids than on other epidermal cells
5) = guard cells with ± no crystalloids

VD/D/L = very dense/dense/loose wax layer
Shaded = membraneous platelets with lobed margins
⟷ = wax dimorphism (in *G. campanulata* 2 different sizes of non-entire platelets)
underlined = different wax types on upper (ad) and lower (ab) leaf surface
( ) = unpublished names of species or subspecies

versely ridged rodlets on epidermal and guard cells, sometimes hollow and with irregular cross sections (Figure 6, arrows).

G. undulata Herb.: Smooth layer as in *G. barkerae* D.Müll.-Doblies, *G. britteniana* Baker (Figure 1) and other species mentioned (see above).

G. uteana D.Müll.-Doblies (Figures 11 and 12): Conspicious differentiation of epidermal cells. Leaf margins with isodiametric papilla-like cells and without cuticular foldings, inner cells ± tabulated and with striking cuticular foldings (Figure 11) (Weiglin in prep.). Dense layer of membranous platelets with lobed margins as in *G. ciliaris* (Thunb.) Thunb., *G. hallii* D.Müll.-Doblies and *G. heinzeana* D.Müll.-Doblies (Figure 4) (see above), somewhat degraded, only at leaf margins (Figure 12); median part of leaf with smooth layer (wax dimorphism) (Figure 11).

G. uteana D.Müll.-Doblies (subsp. *glauca,* U.&D.M-D 82139): Very dense layer of membranous platelets on epidermal and guard cells as in *G. afra* L., *G. linearis* L. Bolus and *G. multifolia* L. Bolus (Figure 3) (see above).

G. verrucosa Marl.: Larger membranous platelets and distinctly smaller entire platelets, the latter forming a dense layer on epidermal and guard cells (wax dimorphism) (Figure 10).

G. verticillata R. Br. ex Herb. (Figure 7): Loose layer of entire platelets on epidermal and guard cells, distinctly oriented into rosettes, each consisting of 5–15 platelets, as in *G. afra L.* (subsp. *philosphica*, U.&D.M-D 79236), *G. lata* L. Bolus, *G. roggeveldensis* D.Müll.-Doblies (see above) and (*G. vogeliana*, U.&D.M-D 88112) (see below).

G. villosa (Thunb.) Thunb.: Loose layer of very small entire platelets with transitions to granules on epidermal and guard cells with slight tendency to parallel orientation as in *G. namaquensis* (Schonl.) Oberm. and *G. setosa* Marl. (Figure 8) (see above).

(*G. vogeliana*, U.&D.M-D 88112): Dense layer of non-entire platelets on epidermal and guard cells, distinctly oriented into rosettes, each consisting of 5-15 platelets, as in *G. afra L.* (subsp. *philosphica*, U.&D.M-D 79236), *G. lata* L. Bolus, *G. roggeveldensis* D.Müll.-Doblies and *G. verticillata* R. Br. ex Herb. (Figure 7) (see above).

Discussion

To date the most comprehensive survey of epicuticular waxes among the Monocotyledons (Frölich and Barthlott 1988) shows the Amaryllidaceae as having manifold and widespread wax-types. Rare wax-types, however, were also observed as terete rodlets in *Cyrtanthus purpureus* (Ait.) Traub and *Leucojum aestivum* L. or as transversely ridged rodlets in *Brunsvigia josephinae* Ker-Gawl. Frölich and Barthlott (1988) assign a relatively isolated position for the family bearing in mind that the pronounced and systematically relevant '*Convallaria*-wax-type' (parallel oriented platelets) does not occur in this family. The Amaryllidaceae, however, are firmly embedded in the order Asparagales of the Lilianae (Dahlgen, Clifford and Yeo 1985, Duvall *et al.* 1993, Chase *et al.* 1995, Rudall and Cutler 1995) and, furthermore, is closely related to Alliaceae and Agapanthaceae both morphologically and at the molecular level (Dahlgen, Clifford and Yeo 1985, Chase *et al.* 1995, Fay *et al.* 1994, 1995, Fay and Chase 1996, Ito *et al.* 1999, Meerow *et al.* 1999). Therefore, instead of being isolated, the family should rather be described as being divergent when regarding the structure of its epicuticular wax crystalloids.

Frölich and Barthlott (1988) did not investigate the wax-types of *Gethyllis*. Thus, this investigation represents the very first attempt. From the results of this study of epicuticular wax types it is evident that the genus *Gethyllis* itself is not uniform and warrants further investigation (Weiglin in prep.). The results in Table 2 show a high diversity of the epicuticular wax ultrastructure within the genus *Gethyllis*. In 12

species non-entire platelets were observed, nine species have membranous platelets, seven species possess entire platelets, eight species smooth layers and only *G. transkarooica* is distinguished by its transversely ridged rodlets. No variations within the wax types of populations from different localities could be established. This is in agreement with the general assumption that wax types, generally, are geneticall y fixed (cf. Cutler and Brandham 1977, Barthlott and Wollenweber 1981, Cutler 1982, Barthlott *et al.* 1998). In three species, however, a wax dimorphism (but also no obvious differences between different populations) was discovered; either within one cell surface (*G. campanulata*, *G. verrucosa*) or between the leaf periphery and middle of the leaf (*G. uteana*).

The wax types between species and (unpublished) subspecies in 1) *G. afra* and *G. afra* (subsp. *philosphica*, U.&D.M-D 79236) and 2) *G. uteana* and *G. uteana* (subsp. *glauca*, U.&D.M-D 82139) were found to be different. Therefore, from the evidence of wax morphology, the creation of the two new subspecies is conceivable. Only in the second member of these species, however, could additional micromorphological evidence for two infraspecific taxa be found. This will be discussed in greater detail in a forthcoming paper. Whereas in *G. uteana* the epidermal cells in the middle of the leaf show conspicious cuticular striations, in *G. uteana* (subsp. *glauca*, U.&D.M-D 82139), no striations were found (Weiglin in prep.). In *G. afra and G. afra* (subsp. *philosphica*, U.&D.M-D 79236), however, no such differentiation occurs. Five species with non-entire platelets (*G. afra* (subsp. *philosphica*, U.&D.M-D 79236), *G. lata*, *G. roggeveldensis*, *G. verticillata*, (*G. vogeliana*, U.&D.M-D 88112) and one species with entire platelets (*G. cavidens*) show a somewhat rosette-like orientation which was first discovered in its typical form in the order Fabales and later in some other closely related families (Ditsch *et al.* 1995, Ditsch and Barthlott 1997). In *Gethyllis* the similarity to this 'Fabales-type' orientation seems to have a convergent origin. The highly significant '*Convallaria*-wax-type' which characterizes the superorder Lilianae (Barthlott and Frölich 1983, Frölich and Barthlott 1984, Barthlott 1990, 1994, Theisen and Barthlott 1998, Barthlott *et al.* 1998) was not found in its typical form which confirms the previous results of Frölich and Barthlott (1988). However, in four species (*G. campanulata*, *G. namaquensis*, *G. setosa* and *G. villosa*) a slight tendency to parallel orientation of non-entire or entire platelets could be assigned.

The author is aware of the fact that epicuticular wax morphology represents only a single character and that a systematic evaluation cannot be based on this character alone. Also, the chemical components of the waxes in *Gethyllis* are, to date, unknown. Thus, the wax chemical composition of a species could prove to be decisive for determining its final systematic and/or taxonomic position (Baker 1982, Jeffree 1986, Meusel *et al.* 1994, Barthlott *et al.* 1998). At first glance it appears that the wax ultrastructure findings are not consistent with other findings for vegetative, floral and genetic characters (Burtt 1970, Müller-Doblies 1982, 1986, Vosa 1986, Meerow and Snijman 1998, Snijman 2000). For instance, the diploid chromosome number is invariably 12 (Vosa 1986) and the flowers are fugacious and don't have many reliable taxonomical characters (Müller-Doblies 1986, Snijman 2000). Furthermore, the previous attempts to subdivide the genus cannot be supported by the present results. The sections *Orthostylis* (*G. verticillata* and *G. villosa*) and *Clinostylis* (*G. longistyla*) of H. Bolus (MacOwan and Bolus 1881) all have entire platelets. Species with more than six stamens (*G. afra*, *G. hallii*, *G. campanulata*, *G. multifolia*, *G. grandiflora*, *G. britteniana*, *G. namaquensis*, *G.ciliaris*) and also species with six stamens, used by Baker (1885) as a character in his key to the species and by Müller-Doblies (unpubl.) to distinguish between the supposed subgenera, also have different wax types. There appears to be a weak correlation between species with 2-armed (T-shaped) trichomes (*G. villosa*, *G. longistyla*, *G. verrucosa*, (*G. calcicola*, U.&D.M-D 84028), *G. barkerae*, *G. setosa*) and species with entire platelets (*G. villosa*, *G. longistyla*, *G. verrucosa* and *G. setosa*). Within this group only *G. barkerae* and (*G. calcicola*, U.&D.M-D 84028) have no epicuticular wax crystalloids (smooth layer).

The occurrence of transversely ridged rodlets prevalent only in *Gethyllis transkarooica* is unique for this genus which may imply that it has a relatively isolated position within this genus. On the other hand, a convergent origin as was also suggested for other plants, i.e. the occurrence of the '*Strelitzia*-wax-type' in *Dracaena* L. (cf. Frölich and Barthlott 1988, Barthlott and Theisen 1998) should also be taken into account. One cannot favour any of the two hypotheses as yet but, in the Amaryllidaceae, transversely ridged rodlets are, based on our current knowledge, confined *to Brunsvigia josephinae* (Frölich and Barthlott 1988) and a few other taxa within the Lilianae (Barthlott 1990, Barthlott and Theisen 1998). Further investigation is needed in order to clarify the taxonomic and phylogenetic interrelations of the genus *Gethyllis*.

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